

1 **Diversity in rest-activity patterns among Lake Malawi cichlid fishes suggests novel**
2 **axis of habitat partitioning**

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11

12 **Abstract**

13 Animals display remarkable diversity in rest and activity patterns that are regulated by
14 endogenous foraging strategies, social behaviors, and predator avoidance. Alteration in
15 the circadian timing of activity or the duration of rest-wake cycles provide a central
16 mechanism for animals to exploit novel niches. The diversity of the 3000+ cichlid species
17 throughout the world provides a unique opportunity to examine variation in locomotor
18 activity and rest. Lake Malawi alone is home to over 500 species of cichlids that display
19 divergent behaviors and inhabit well-defined niches throughout the lake. These species
20 are presumed to be diurnal, though this has never been tested systematically. Here, we
21 measure locomotor activity across the circadian cycle in 12 cichlid species from divergent
22 lineages and distinct habitats. We document surprising variability in the circadian time of
23 locomotor activity and the duration of rest. In particular, we identify a single species,
24 *Tropheops* sp. “red cheek” that is nocturnal. Nocturnal behavior was maintained when
25 fish were provided shelter, but not under constant darkness, suggesting it results from
26 acute response to light rather than an endogenous circadian rhythm. Finally, we show
27 that nocturnality is associated with increased eye size, suggesting a link between visual
28 processing and nighttime activity. Together, these findings identify diversity of locomotor
29 behavior in Lake Malawi cichlids and provide a system for investigating the molecular and
30 neural basis underlying the evolution of nocturnal activity.

31

32 **1. Introduction**

33 Animals display remarkable diversity in rest and activity patterns. Circadian differences in
34 locomotor activity and rest can differ dramatically between closely related species, or
35 even between individuals of the same species, raising the possibility that it can be
36 adaptive and subject to selection[1–3]. Indeed, circadian regulation of locomotor activity
37 is strongly associated with foraging strategies, social behaviors, and predator avoidance
38 that are critical factors in organismal fitness[4,5]. Alteration in the circadian timing of
39 activity or the duration of rest-wake cycles provide a central mechanism for animals to
40 exploit novel niches.

41

42 Across phyla the timing of rest and activity is regulated by a circadian clock that persists
43 under constant conditions, as well as acute response to environmental cues that include
44 light and food availability[6]. For example, many teleost species display robust diurnal
45 locomotor rhythms including the goldfish (*Carassius Auratus*), the Mexican tetra
46 (*Astyanax mexicanus*), and the zebrafish (*Danio rerio*)[1,7,8]. Conversely, limited
47 examples of nocturnal teleosts have been identified including the plainfin midshipman,
48 the Senegalese sole, and the doctor fish, *Tinca tinca* [9–11]. Despite these conspicuous
49 differences, variation in rest and activity patterns have not been well described within a
50 lineage. Moreover, the ecological basis of such variation, and its relationship to niche
51 exploitation has not been studied systematically.

52

53 Cichlids represent a leading model for investigating the evolution of development,
54 morphology, and complex behavior. In Lake Malawi alone, there are over 500 species of

55 cichlids, inhabiting a diversity of environmental and feeding niches. Cichlid species exhibit
56 a high degree of habitat fidelity and partition their environment along discrete ecological
57 axes, including distinct biotic (food availability, predation, and parasites) and abiotic (light,
58 water chemistry) environments that play a critical role in the origins and maintenance of
59 cichlid biodiversity[12–17]. Predation on Malawi cichlids is considered to be relatively low,
60 which is thought to have contributed to their evolutionary and ecological success[18].
61 However, the lake is home to predators, including the Cornish jack *Mormyrops*
62 *anguilloides*, that feed on cichlids in the intermediate and near-shore rocky habitat. *M.*
63 *anguilloides* are weakly electric fish that hunt at night using electrical pulses thought to
64 be undetectable by cichlids[19]. Field studies on this predatory behavior have suggested
65 that cichlids are largely diurnal[19], in agreement with the notion that rest represents a
66 form of adaptive inactivity that allows for predator avoidance[20]. Deviations from diurnal
67 activity have been noted for new world cichlids, which exhibit nocturnal parental care of
68 eggs[21,22], and the ability of some Malawi cichlids to forage in low-light conditions, via
69 widened lateral line canals, suggests the potential for nocturnal behaviors to evolve in this
70 group[23]. Given that Malawi cichlids exhibit an impressive magnitude of diversity in an
71 array of anatomical and behavioral traits, we reasoned that they may also exhibit variation
72 in rest-activity patterns. Indeed, this could represent an important, but underappreciated,
73 dimension of habitat partitioning.

74

75 The development of automated tracking of locomotor activity in fish species has been
76 applied for the study of sleep and locomotor activity in zebrafish and Mexican
77 cavefish[24]. These methodologies provide the opportunity for comparative approaches

78 that examine differences in activity between populations, and across contexts. Here, we
79 extend this methodology to study sleep across 12 species of cichlids, from diverse
80 habitats. Our choice of species focuses on the near-shore rock-dwelling clade of Malawi
81 cichlids (i.e., *mbuna*), but we also include representative species from other major
82 lineages. Our goal is not to characterize the evolution of rest-activity patterns *per se*, but
83 rather to better understand the degree and type of variation exhibited by this group. We
84 identify robust variation in the quantity, as well as the circadian timing, of rest and activity.
85 In addition, this analysis reveals, for the first time, a nocturnal species of Malawi cichlid,
86 suggesting that circadian regulation of activity may provide a mechanism for niche
87 exploitation in African cichlids. Together, these findings suggest cichlids can be used as
88 a model to study the evolution of molecular mechanisms for variation in locomotor
89 rhythms.

90

91 **2. Materials and Methods**

92 *(a) Fish stocks and husbandry*

93 Cichlids used for experiments were reared following standard protocols approved by the
94 University of Massachusetts Institutional Animal Care and Use Committee. Cichlids
95 were housed in the Albertson fish facilities at a water temperature of 28.5°C, kept on a
96 14:10 hour light-dark cycle, and fed a diet of a flake mixture consisting of ~75% spirulina
97 algae flake and ~25% yolk flake twice a day. Cichlids were derived from wild-caught
98 animals that were either reared in the Albertson fish facilities (*Labeotropheus*
99 *trewavasae*, *Maylandia zebra*, *Tropheops* sp. “red cheek”), or obtained through the
100 aquarium trade (*Sciaenochromis fryeri*, *Copadichromis trewavasae*, *Aulonocara*

101 *stuartgranti*, *Dimidiochromis compressiceps*, *Labeotropheus fuelleborni*, *Iodotropheus*
102 *sprengerae*, *Tropheops* sp. “red fin”, *Tropheops* sp. “elongatus Boadzulu”). Because of
103 the nature of the testing tanks (see below), all fish were tested at the late juvenile stage,
104 making sex determination difficult to assess at the time; however, after the experiments
105 took place, stocks were grown out and it could be confirmed that sex ratios were 50:50
106 on average.

107

108 (b) *Behavioral analysis*

109 24 hours prior to the beginning of each experiment, juvenile fish were transferred from
110 their home tanks into 10L tanks (Carolina Biologicals) with custom-designed partitions
111 that allowed for up to 3 fish to be individually housed in each tank. After 24 hours of
112 acclimation, fish were fed, tanks were given a 50% water change to maintain water
113 quality, and behavior was recorded for a 24 hour period beginning at zeitgeber time (ZT)
114 3, 3 hours after light onset. Videos were recorded at 15 frames/second using a USB
115 webcam (LifeCam Studio 1080p HD Webcam, Microsoft) through the video processing
116 software VirtualDub (v1.10.4). To allow for recording during the dark period and provide
117 consistent lighting throughout the day, cameras were modified by removing their IR
118 filters and replacing with IR long-pass filters (Edmund Optics Worldwide), and tanks
119 were illuminated from behind using IR light strips (Infrared 850 nm 5050 LED Strip Light,
120 Environmental Lights).

121

122 For experiments testing the effect of shelter on locomotor activity, a small PVC tube, 3”
123 in length x 1” outer diameter, was added to each chamber at the beginning of the

124 acclimation period. For experiments testing the effect of light, fish were acclimated to
125 their tanks on a normal 14:10 LD cycle, and then recorded in 24 hours of darkness.

126

127 Following acquisition, recordings were processed in Ethovision XT 15 (Noldus) to
128 extract positional data for individual fish throughout the 24 hour period, and this data
129 was used to calculate velocity and locomotor activity, as previously described[25].

130

131 *(c) Analysis of activity and rest*

132 To identify variation in rest and activity patterns across cichlid species, positional data
133 was exported from Ethovision and analyzed using a custom-made Perl script (v5.10.0)
134 and Excel Macro (Microsoft). A threshold of 4 cm/sec was set to correct for passive drift
135 of the animal; any reading over this threshold was classified as active swimming and
136 used to calculate velocity. Any period of inactivity lasting greater than 60 seconds was
137 classified as a “rest” bout, and the time and duration of each rest bout was recorded to
138 generate profiles of rest throughout the day.

139

140 *(d) Measurements of eye size*

141 Fish were imaged using a digital camera (Olympus E520) mounted to a camera stand.
142 All images included a ruler. Using the program Image J[26], measures of standard
143 length, head length, and eye area were obtained for each fish. Eye size was measured
144 in fish used in the behavioral analysis. In addition, when possible, we augmented these
145 samples with wild-caught animals from the Albertson lab collections. In particular, we
146 added wild-caught samples to the *L. fuelleborni*, *M. zebra*, *T. sp. “red cheek”*, and *T. sp.*

147 “red fin” populations. Two measures of eye-size were obtained: total area, and area
148 relative to head length. Because relative eye-size exhibits strong allometric
149 effects[27],residuals were obtained via a linear regression of eye-size on standard
150 length in R[28]. All statistical analyses were based on residuals. Results were the same
151 whether we used absolute eye-size or eye-size relative to head length. We therefore
152 only present data using absolute eye-size.

153

154 (e) *Statistics and analysis*

155 One-Way ANOVAs were carried to identify inter-species differences in overall locomotor
156 activity, average waking velocity, rest duration, and total time in shelter between
157 species. To identify differences between multiple conditions, such as activity in the light
158 vs. dark, or shelter vs no-shelter conditions, a Two-Way ANOVA was carried out, and
159 followed by Sidak’s multiple comparisons *post-hoc* test. To identify significant rhythms in
160 activity across the day-night cycle, an “activity change ratio” was calculated as follows:
161 First, average hourly day- and night-time activity were calculated for each fish. Night-
162 time activity was subtracted from day-time activity, and the result was divided by their
163 sum, providing a normalized day/night preference score. To identify significant
164 rhythmicity, one-sample t-tests were performed. To identify differences between the
165 *mbuna* and non-*mbuna* groups, nested ANOVAs were performed. All statistical
166 analyses were carried out using InStat software (GraphPad Prism 8).

167

168 **3. Results**

169 *(a) Variation in activity and rest behaviors*

170 To measure variation in activity across Lake Malawi cichlids, we compared the locomotor
171 activity in twelve different species, across eight genera, of cichlids that were selected for
172 diversity in habitat, behavior, and lineage representation. We sampled more deeply in the
173 rock-frequenting *mbuna* clade (n=7 species; n=4 genera), which occupy a complex, three-
174 dimensional habitat characterized by a high density of cichlid individuals (Fig 1A). In
175 addition, we analyzed activity patterns in four non-*mbuna* species, which occupy the
176 intermediate to open-water habitat (Fig 1B). Following an initial 24 hour period of
177 acclimation, activity was recorded in individually housed juvenile fish across 24 hours in
178 standard light-dark conditions, with infrared lighting used to monitor locomotor activity
179 during the night as previously described in *A. mexicanus*[25]. Quantification of total
180 locomotor activity over 24 hours identified marked variation across species, with certain
181 species (i.e., *S. fryeri*) exhibiting significantly lower activity than all other species tested,
182 while the activity of others (i.e., *T. sp. "elongatus Boadzulu"*) was significantly greater than
183 all other species (Fig 1C). Notably, variation in mean activity was continuously distributed
184 between these two extremes. In addition, there was a division between *mbuna* and non-
185 *mbuna* species, with *mbuna* species trending towards increased locomotion relative to
186 non-*mbuna* species ($p = 0.052$).

187

188 To determine whether these differences were due to hyperactivity or differences in rest,
189 we measured the average waking velocity for each population. Among all species tested,

190 only one (*T. sp. "elongatus* Boadzulu"), displayed significantly higher swimming velocity,
191 suggesting the bulk of the variation among species is due to differences in rest/activity
192 regulation (Fig 1D). In agreement with this notion, there were significant inter-species
193 differences in the duration of rest bouts lasting greater than one minute (Fig 1E). The
194 majority of species displayed very little rest, averaging less than 3 hours/day, while three
195 species, *C. trewavasae*, *D. compressiceps*, and *S. fryeri* (all non-*mbuna*) spent
196 significantly longer resting than all other species tested. The average rest duration of *S.*
197 *fryeri* was over 10-fold different than other species tested. Together, these findings
198 suggest that differences in total locomotor activity between cichlid species are largely
199 attributable to differences in rest. Notably, *mbuna* species together rested significantly
200 less than non-*mbuna* species (Fig 1E), possibly reflecting adaptation to the near-shore
201 rocky habitat. Support for this possibility, as opposed to historical contingency, is the
202 observation that *A. stuartgranti*, a non-*mbuna* species that co-occurs with *mbuna*, rests
203 less than other non-*mbuna* species (Fig 1E).

204

205 *(b) Variation in patterns and magnitudes of rhythmic activity*

206 To determine whether there are differences in circadian modulation of activity, we
207 compared activity over the light-dark cycle (Fig 2A). We found evidence for strong diurnal
208 activity in three *mbuna* species (*L. fuelleborni*, *T. sp. "elongatus* Boadzulu", and *I.*
209 *sprengerae*), while activity did not significantly differ based on light or dark phases in
210 seven species tested (Fig 2B). A single species, *Tropheops* sp. "red cheek", was
211 significantly more active in the night, providing the first evidence for nocturnality in a Lake
212 Malawi cichlid (Fig 2B). To account for variation in total locomotion between fish of

213 different species, we quantified preference for light and dark activity for each individual
214 tested. In agreement with quantification of average locomotor activity, *T. sp. "red cheek"*
215 had significantly greater preference for nighttime activity while *L. fuelleborni*, *T. sp.*
216 "elongatus Boadzulu", and *I. sprengerae*, had significantly greater preference for daytime
217 activity (Fig 2C). This analysis also suggests a preference for diurnal activity in *L.*
218 *trewavasae*, and for nocturnal activity in two additional non-*mbuna* species (*C.*
219 *trewavasae* and *S. fryeri*).

220

221 In other diurnal teleosts, such as *A. mexicanus* and *D. rerio*, rest is largely consolidated
222 during nighttime. To quantify time of day differences in rest across cichlid species, we
223 compared the average amount of rest per hour across the 14 hours of light and 10 hour
224 dark periods (Fig S1). This analysis is largely in agreement with analysis of locomotor
225 activity, with day-active species consolidating rest during the dark period, and *vice versa*.

226

227 Since *T. sp. "red cheek"* is a highly territorial and aggressive species[29,30], it is possible
228 that the nighttime activity of this species represents a search strategy for locations that

229 provide shelter from predators, as opposed to a natural reflection of activity patterns. To
230 differentiate between these possibilities, we provided each animal with a 3 inch cylindrical
231 shelter (PVC piping), and measured behavior across light and dark conditions (Fig 3A).

232 We analyzed the total activity across the circadian cycle, as well as time spent in the
233 shelter in *T. sp. "red cheek"*, as well as in *L. trewavasae* and *M. zebra*, closely related
234 *mbuna* species that co-occur with *T. sp. "red cheek"*. These two species also exhibited
235 lower and indistinguishable activity levels during the day and night, and we were

236 interested to see if the addition of shelter would alter this pattern. When provided a hiding
237 spot, *T. sp.* “red cheek” remained robustly nocturnal, while *M. zebra* and *L. trewavasae*
238 did not show light dark preference, which is consistent with their activity patterns in the
239 absence of shelter (Fig 3B). We quantified the total time animals spent within the shelter
240 and found that *L. trewavasae* spent significantly more time in the shelter than *M. zebra*
241 and *T. sp.* “red cheek” (Fig 3C), which is consistent with this species’ behavior in the wild.
242 *L. trewavasae* has an elongated and dorsal-ventrally compressed body plan, and exhibits
243 habitat preference for cracks and crevices in the wild[30,31]. Further, *L. trewavasae* spent
244 more time in the shelter during the night period, consistent with an increased need to
245 avoid nocturnal predators (Fig 3D). Conversely, there were no differences in shelter
246 preference between light or dark periods for *M. zebra* and *T. sp.* “red cheek”. Together,
247 these findings suggest that the presence of a shelter does not significantly impact the
248 activity pattern of the cichlid species tested, and that the nocturnal locomotor activity of
249 *T. sp.* “red cheek” does not represent a search for shelter.

250

251 It is possible that the nocturnal locomotor behavior of *T. sp.* “red cheek” is due to an
252 endogenous circadian rhythm or a differential response to light. To distinguish between
253 these possibilities, we measured locomotor activity under constant dark conditions.
254 Briefly, fish were acclimated under standard 14:10 light dark conditions, then activity was
255 recorded for 24 hours under constant darkness (Fig 4A). While *T. sp.* “red cheek” are
256 significantly more active during the dark period under light:dark conditions, there was no
257 difference between light and dark activity under constant darkness. (Fig 4A). A
258 comparison of total activity between the day (with light present) and the subjective day

259 (darkness) reveals that activity is significantly lower in the presence of light (Fig 4B).
260 These findings are consistent with a role for light in suppressing activity, thereby inducing
261 nocturnal behavior.

262

263 *(c) Activity is higher in territorial species*

264 General information regarding each species', habitat, behavior, prey-preference, and
265 phylogenetic relationship is provided in Table 1. To determine whether any variables of
266 rest or activity associate with these ecological factors, we compared locomotor data with
267 known ecological variables. Unsurprisingly, species described as territorial exhibited, on
268 average, greater overall activity compared to those characterized as weakly or non-
269 territorial. We note, however, that any conclusion about the relationship between
270 locomotor activity and ecology/phylogeny may be premature, as significant differences in
271 rest-activity behavior exist between closely related and ecologically similar species (e.g.,
272 within *Tropheops* and *Labeotropheops*). The more general conclusion to be drawn from
273 these data is that Lake Malawi cichlids exhibit substantial and continuous variation in
274 activity levels and patterns.

275

276 *(d) Eye size is associated with night-time activity*

277 Across fish species, nocturnality or adaptation to low-light conditions is associated with
278 larger eye size. In addition, species that rely on visual modes of foraging generally
279 develop larger eyes[32–34]. On the other hand, species adapted to forage on attached
280 algae generally possess smaller eyes, consistent with a functional tradeoff for the
281 production of power during jaw closure[35]. Specifically, algal scrapers tend to exhibit

282 smaller and dorsally shifted eyes to accommodate larger adductor muscles that are
283 situated below the eyes[36]. To understand how eye size relates to these variables, we
284 measured eye size in cichlid individuals in all species tested (Fig 5A, 5B), and tested for
285 significant correlations. Notably, we did not observe an obvious association between eye
286 size and lineage or foraging mode (Fig 5B). While the visual hunting species *C.*
287 *trewavasae* and *S. fryeri* possess larger eyes on average, *D. compressiceps*, an ambush-
288 hunter, has the smallest eyes of the species measured. Likewise, while the algal scraping
289 species within the genus *Labeotropheus* has relatively small eyes, the attached algae
290 specialists, *T. sp.* “red cheek”, has the largest relative eye size of the species measured.
291 The other species with large eyes was *A. stuartgranti*, which is a sonar hunter with
292 enlarged lateral line canals capable of foraging in low-light conditions[23]. Neither did we
293 identify a correlation between rest amount and eye size (Fig 5C). However, there was a
294 strong correlation between eye size and preference for night-time activity (Fig 5D).
295 Whether the large eye size in these species represents an adaptation to nocturnality
296 remains to be tested, but it is a notable morphological correlate worthy of further
297 investigation.

298

299 **4. Discussion**

300 The diversity of the ~3000 cichlid species throughout the world provides a unique
301 opportunity to examine the effects of ecological niche and evolutionary history on the
302 regulation of locomotor activity and rest. Cichlid species have undergone adaptive
303 radiations, resulting in morphologies and behaviors that can be highly specialized to
304 specific ecological niches. The ecology inhabited by cichlids includes species with habitat

305 fidelity to shorelines, deep water, and the intermediate zone between rocky and sandy
306 regions. In addition, many species are generalists and inhabit multiple different niches.
307 Here we focused our analysis on Lake Malawi cichlids, that alone likely contains over 500
308 species of fish, many of which share overlapping ecological niches[37]. The well
309 characterized ecosystem within the lake, as well as the taxonomic diversity uniquely
310 positions cichlids for investigating the role of ecology in shaping behavioral evolution.
311 Indeed, an important outstanding question is how can so many species with dietary
312 overlap co-exist in this lake? Many factors have been proposed to contribute including
313 the multitude of ecological resources available in this large tropical lake, low predation,
314 as well as the ability of cichlid species to evolve highly specific courtship and feeding
315 behaviors[18,38]. Circadian regulation of activity and rest may provide an additional
316 contributor to niche partitioning, reproductive isolation and even speciation, yet these
317 behaviors have not been studied systematically. The finding that the timing and duration
318 of rest and activity varies dramatically, and continuously, between populations of Lake
319 Malawi cichlids suggest this is a fruitful line of inquiry.

320

321 While circadian rhythms have been studied in detail across many different animal species,
322 surprisingly little is known about the presence and regulation of free running rhythms in
323 teleosts. For example, Nile tilapia, *O. niloticus*, display extreme variability under light-dark
324 conditions that ranges from diurnal to nocturnal, yet the majority of animals maintain
325 rhythms of ~24hrs under constant dark conditions[39]. Feeding is likely a critical mediator
326 of activity rhythms, though in some species, the daily timing of feeding differs from
327 locomotor activity. For example, zebrafish are highly diurnal and maintain 24 hour

328 rhythms, yet feeding occurs primarily during the night[40]. A similar trend has been noted
329 in cichlids, where diurnal species exhibit mating and brooding behaviors primarily at
330 night[21,41]. These findings suggest a high degree of flexibility in the circadian regulation
331 of behavior, and that the circadian timing of many behaviors may differ from locomotor
332 behavior that is typically used as a primary readout of the circadian clock[42]. Here, we
333 focused specifically on locomotor activity and did not provide social conspecifics or food
334 that could influence the timing of activity. Fully understanding the evolution circadian
335 behavior of each species and its relationship to its natural environment will require
336 examining additional behaviors that may be under circadian regulation.

337

338 A notable finding from this study is a species that appears to be nocturnal. *Tropheops* sp.
339 “red cheek” is a member of a highly speciose and ecologically diverse lineage[13,30,43].
340 It is a vigorously territorial species that occupies the near shore rocky habitat, where
341 males defend large patches of rocks, cultivating algae gardens that they only allow
342 potential mates to feed from. This species exhibits significant habitat and dietary overlap
343 with *L. fuelleborni*, another algae foraging species from the rocky shallows. *L. fuelleborni*
344 is arguably one of the most ecologically successful species in the lake, with numerous
345 anatomical adaptations that enable it to dominate this niche[31,44–46]. How then might
346 another species coexist with such a well-adapted forager? Based on the results presented
347 here, it is tempting to speculate that *L. fuelleborni* and *T. sp.* “red cheek” are partitioning
348 their habitat by rest-activity patterns. Consistently, these two species (1) are among the
349 most active of any measured, (2) are both strongly rhythmic, and (3) their rhythmicity is
350 opposite of one another.

351

352 Our findings raise the possibility that *T. sp.* “red cheek” is nocturnal in the wild, and the
353 limited amount of night filming that has been performed in Lake Malawi supports this
354 notion. Specifically, Arnegard and Carlson (2005) documented the nocturnal predatory
355 behavior of the weakly electric species, *M. anguilloides*, on cichlids in the rocky habitat.
356 The footage (available at <https://malawicichlids.com/mw19000.htm>), is impressive and
357 shows the success of the “pack” hunting strategy employed by *M. anguilloides*. Two
358 cichlid species (based on male breeding color) are readily apparent in the footage, *A.*
359 *stuartgranti* and *T. sp.* “red cheek”. Indeed, the very first fish seen in the night footage is
360 a male *T. sp.* “red cheek” (@ 1:15). This fish is not resting within a rocky cave, crack or
361 crevice, but rather it is actively swimming well above the rocks. In fact, in the ~6 minutes
362 of night footage, no fewer than 5 *T. sp.* “red cheek” individuals can be observed, many *A.*
363 *stuartgranti* are observed as well. As a point of comparison, no *Labeotropheus* or
364 *Maylandia* species are readily observed at night, though they are common in the day
365 footage at the beginning and end of the ~8 minute film. This filming was not intended to
366 address questions related to rest-activity patterns in cichlids, and so we are cautious
367 about drawing firm conclusions; however, the trends are conspicuously consistent with
368 our laboratory results.

369

370 It is important to note that our analyses are limited to rest, and we did not examine sleep
371 *per se*. Across phyla, ranging from jellyfish to humans, sleep can be defined by shared
372 behavioral characteristics that include consolidated periods of behavioral quiescence,
373 homeostasis following deprivation and increased arousal threshold, and species-specific

374 posture[47]. In teleosts, the duration of inactivity associated with sleep has been defined
375 as one minute of immobility in larval *A. mexicanus* and zebrafish, and the same duration
376 for adult *A. mexicanus*[48,49]. The duration of sleep and rest is highly variable across
377 many other teleost species, and even between individuals of the same species. For
378 example, different populations of *A. mexicanus* display extreme differences in sleep and
379 activity, with cave dwelling populations of *A. mexicanus* sleeping less than river-dwelling
380 surface fish counterparts. These differences presumably evolved, at least in part, due to
381 increased foraging needs in a nutrient-poor cave environment[50]. Based on previous
382 work in fishes, we defined rest as the total duration of inactivity bouts longer than one
383 minute, and therefore these phenotypes may reflect differences in sleep duration across
384 cichlid species. While specifically examining sleep in cichlids will require defining the
385 period of immobility associated with changes in arousal threshold, posture, poster, and
386 other behavioral characteristics of sleep, we submit that it represents a fruitful line of
387 inquiry as it offers an ideal system in which to delve further into the evolution of sleep and
388 its molecular underpinnings.

389

390 **Data accessibility**

391 Will be determined upon acceptance.

392

393 **Author's contributions**

394 A.C.K and R.C.A conceived the project. E.L. and B.C. ran the sleep experiments. R.C.A
395 measured eye sizes. E.L. analyzed the behavioral and morphological data. A.C.K, E.L.

396 and R.C.A interpreted the results and wrote the manuscript. All authors were involved
397 with rounds of editing and proofing of the figures and manuscript text.

398

399 **Competing interests**

400 The authors declare no competing interests.

401

402 **Funding**

403 This work was supported by the University of Massachusetts and Florida Atlantic
404 University.

405

406 **Acknowledgements**

407 We thank members of the Albertson and Keene laboratories for logistical support with
408 fish care, as well as for thoughtful discussions on various aspects of this project. Ad
409 Konings, Cichlid Press, provided the images of cichlid species in Figure 1. He is
410 acknowledged for his continued and generous support of the cichlid research
411 community through the use of his extensive image library.

412

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562

563 **Table 1**564 General information about the Lake Malawi species under study. Information based on [30,31]. Abbreviations: G =
565 generalist, S = specialist, N = non-territorial, T = territorial, W = weakly territorial, SL = standard length.

566

Genus	species	Clade	Habitat	Depth		Specialist/			
				distribution	Prey Type	Generalist	Territorial	Size (SL)	Distribution
<i>Aulonocara</i>	<i>stuartgranti</i>	Deep Benthic	Intermediate	5-15m	Benthic invertebrates	S	T	<10cm	Lake-wide
<i>Copadichromis</i>	<i>trewavasae</i>	Utaka	Intermediate/open water	10-30m	Zooplankton	S	T	<10cm	Lake-wide
<i>Dimidiochromis</i>	<i>compressiceps</i>	Shallow Benthic	Intermediate with plants	<30m	Small fish/fry	S	W	10-20cm	Lake-wide
<i>Sciaenochromis</i>	<i>fryeri</i>	"Old" sand dweller	Intermediate	10-40m	Small fish/fry	S	W	12-14cm	Lake-wide
<i>Tropheops</i>	sp. "red fin"	<i>mbuna</i>	Rocks with sediment	>10m	Algae/detritus	G	T	<10cm	Lake-wide
<i>Tropheops</i>	sp. "elongatus"	<i>mbuna</i>	Rocks with sediment	>10m	Algae/plankton/	G	T	<10cm	Specific locations, Boadzulu"
<i>Tropheops</i>	sp. "red cheeck"	<i>mbuna</i>	Sediment-free rocks	<10m	Attached filamentous algae	S	T	<10cm	Specific locations, south and north ²
<i>Labeotropheus</i>	<i>trewavasae</i>	<i>mbuna</i>	Between/under rocks with sediment	0-20m	Algae/detritus	G	W	<10cm	Lake-wide
<i>Labeotropheus</i>	<i>fuelleborni</i>	<i>mbuna</i>	Sediment-free rocks	<10m ¹	Attached filamentous algae	S	T	10+cm	Lake-wide
<i>Maylandia</i>	<i>zebra</i>	<i>mbuna</i>	Diverse rocky habitat	5-20m	Loose algae	G	T	<10cm	Lake-wide
<i>Iodotropheus</i>	<i>sprengerae</i>	<i>mbuna</i>	Diverse rocky and intermediate	3-15m ¹	Diverse omnivorous	G	N	<10cm	Southeast

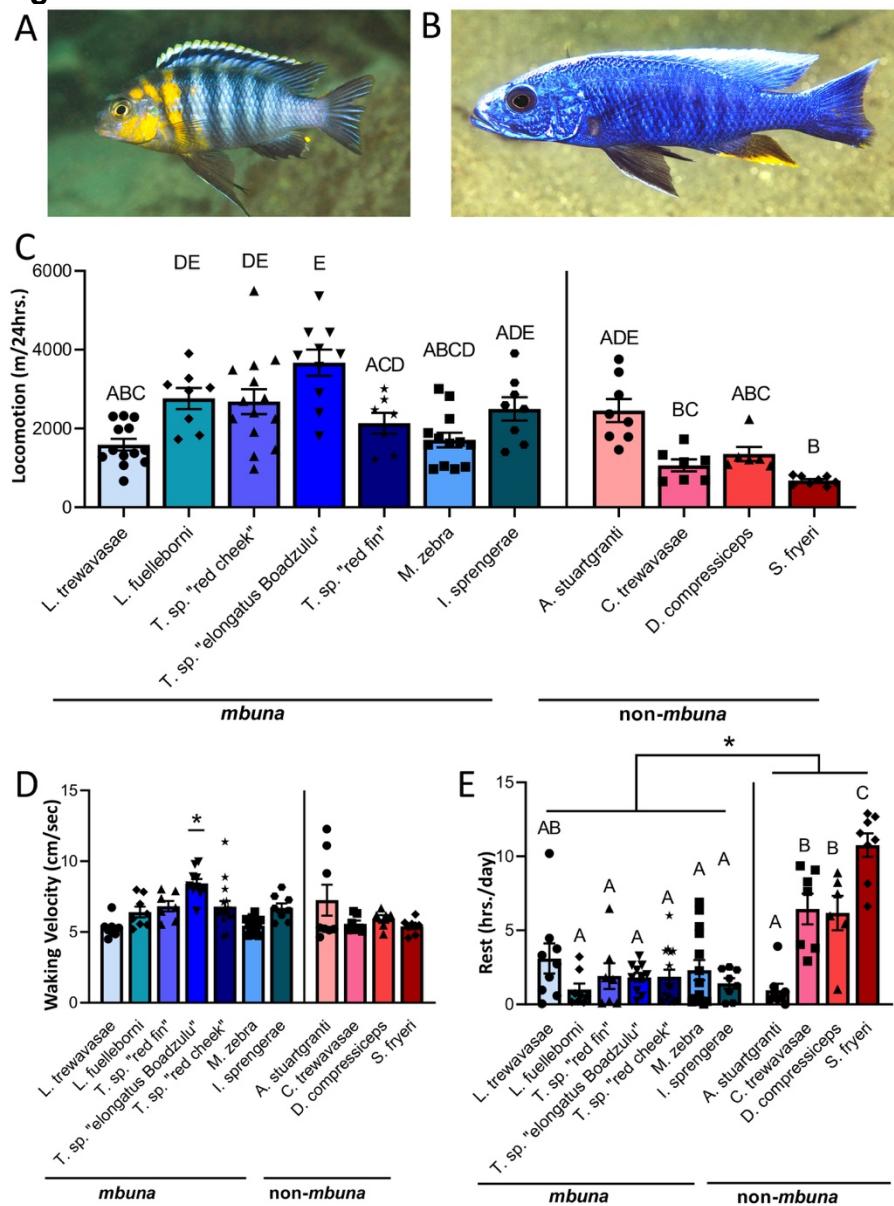
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569 ¹ These species have been observed to penetrate much deeper waters (e.g., ~40m).570 ² This distribution pattern suggests a lake-wide historical distribution.

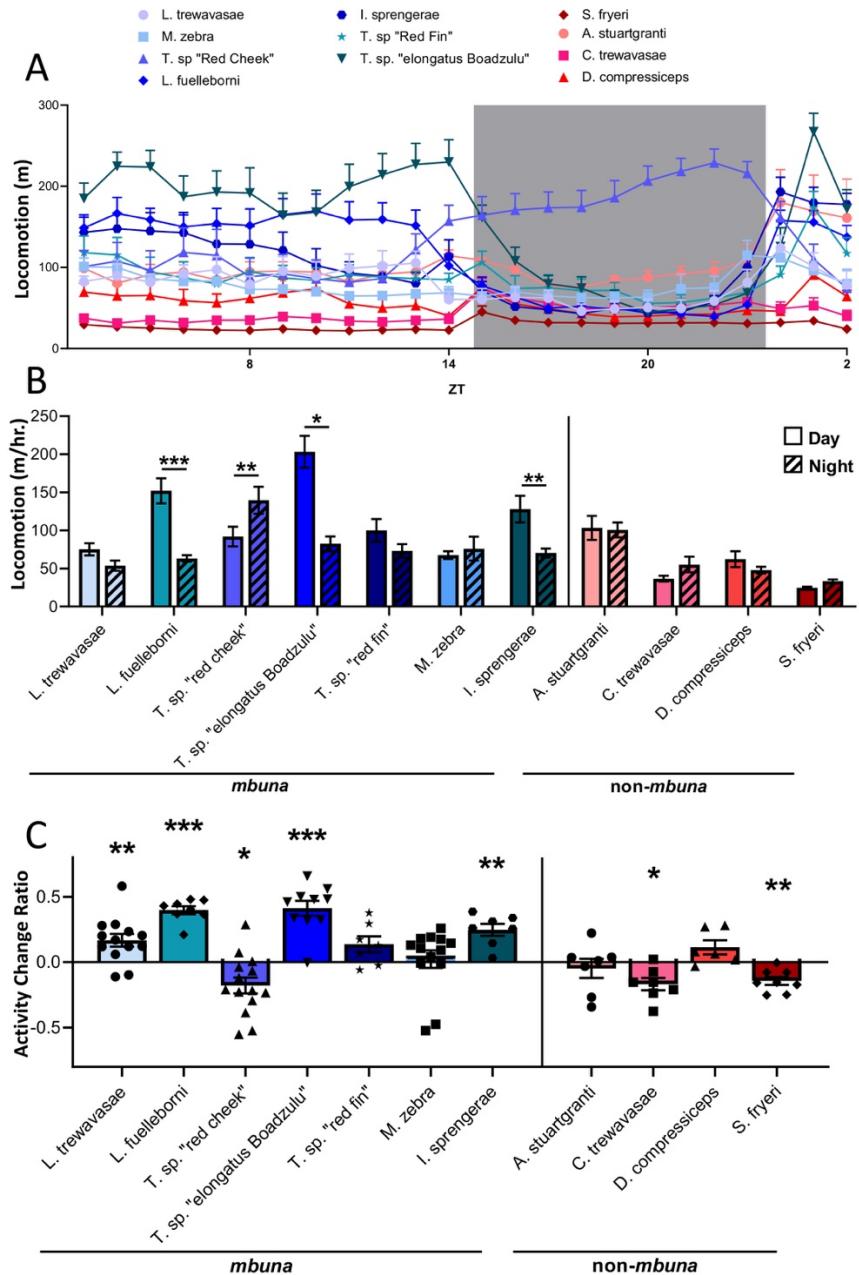
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572 **Figures**



573

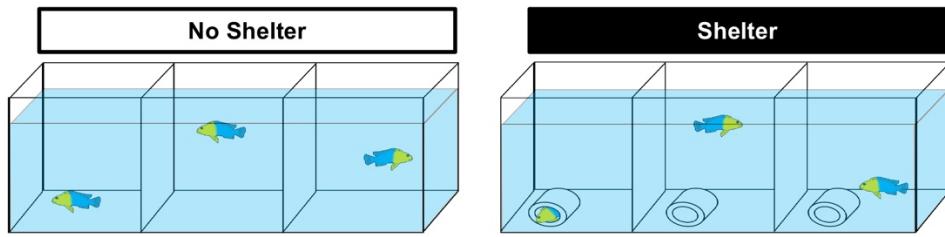
574 **Fig 1.** Evolved differences in locomotor activity between cichlid species. (A) Morphology
 575 of *T. sp. "red cheek"* of the *mbuna* group. (B) Morphology of *S. fryeri* of the *non-mbuna*
 576 group. Images by Ad Konings, Cichlid Press. (C) Total locomotor activity over 24 hours
 577 varies significantly across 11 cichlid species (one-way ANOVA: $F_{10, 91} = 11.10$,
 578 $p < 0.0001$). *Mbuna* species trend towards higher activity than *non-mbuna* species,
 579 although this relationship does not reach significance (nested ANOVA, $F_{1,9} = 5.009$). (D)
 580 Waking velocity over 24 hours is significantly elevated in only one species of cichlid,
 581 *Tropheops* sp. "elongatus Boadzulu" (one-way ANOVA, $F_{10,89} = 5.398$). (E)
 582 Consolidated periods of rest (>60 seconds) vary significantly across *mbuna* and *non-*
 583 *mbuna* groups. (nested ANOVA, $F_{1,9} = 7.808$, $p = 0.0209$).



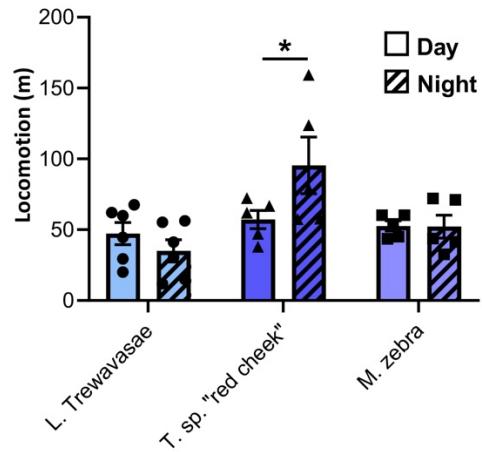
584

585 **Fig 2.** Variation in daily activity rhythms across the day/night cycle. (A) Activity profiles
 586 of all species tested across 24 hours, beginning at ZT3. (B) Several species exhibit
 587 significantly increased activity during the subjective day (*L. fuelleborni*, *T. sp. "elongatus*
 588 *Boadzulu"*, *I. sprengerae*) while a single species exhibits increased locomotor activity
 589 during the subjective night (*T. sp. "red cheek"*) (two-way ANOVA, $F_{10,91} = 13.27$). (C)
 590 Activity change scores, calculated as the difference between daytime and nighttime
 591 activity, divided by their sum, reveal differences in day/night preference across cichlid
 592 species (one-sample t-test; *L. trewavasae* ($t_{12} = 3.419$, $p = 0.0051$), *L. fuelleborni* ($t_7 =$
 593 12.93 , $p < 0.0001$), *T. sp. "red cheek"* ($t_{13} = 2.930$, $p < 0.0117$), *T. sp. "elongatus*
 594 *Boadzulu"* ($t_9 = 7.182$, $p < 0.0001$), *I. sprengerae* ($t_6 = 5.374$, $p = 0.0017$), *C. trewavasae*
 595 ($t_6 = 3.555$, $p = 0.012$), *S. fryeri* ($t_7 = 4.693$, $p = 0.0022$).

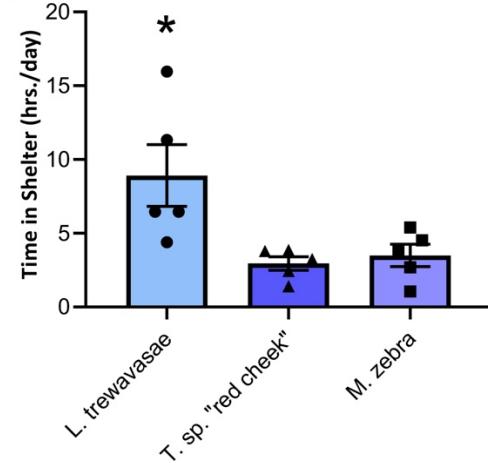
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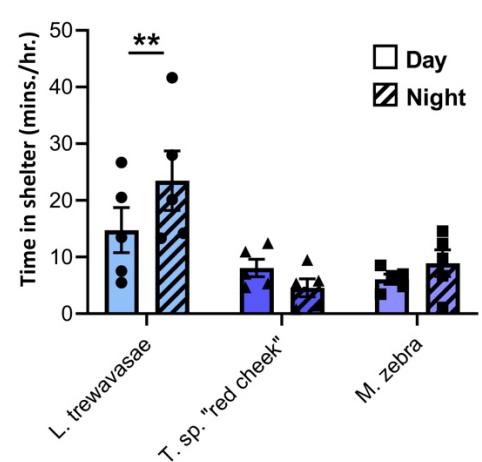
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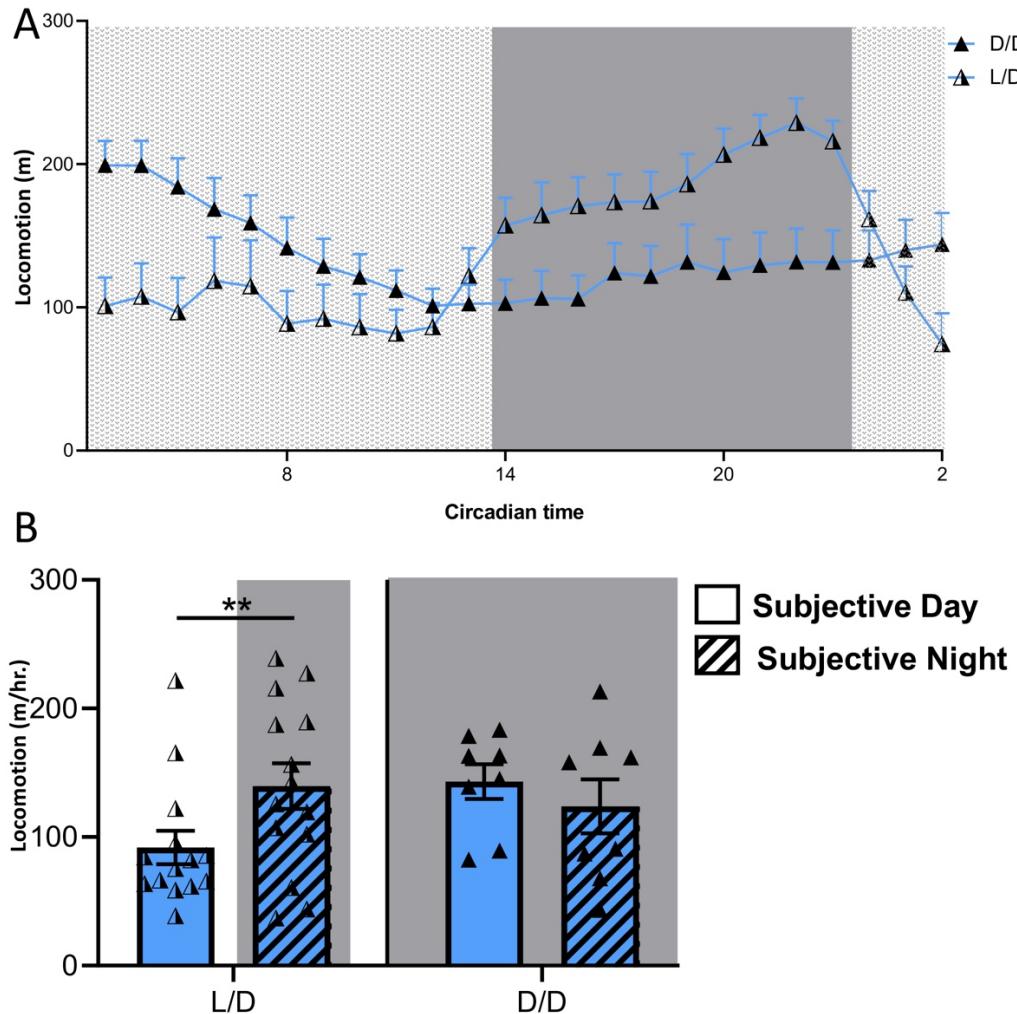


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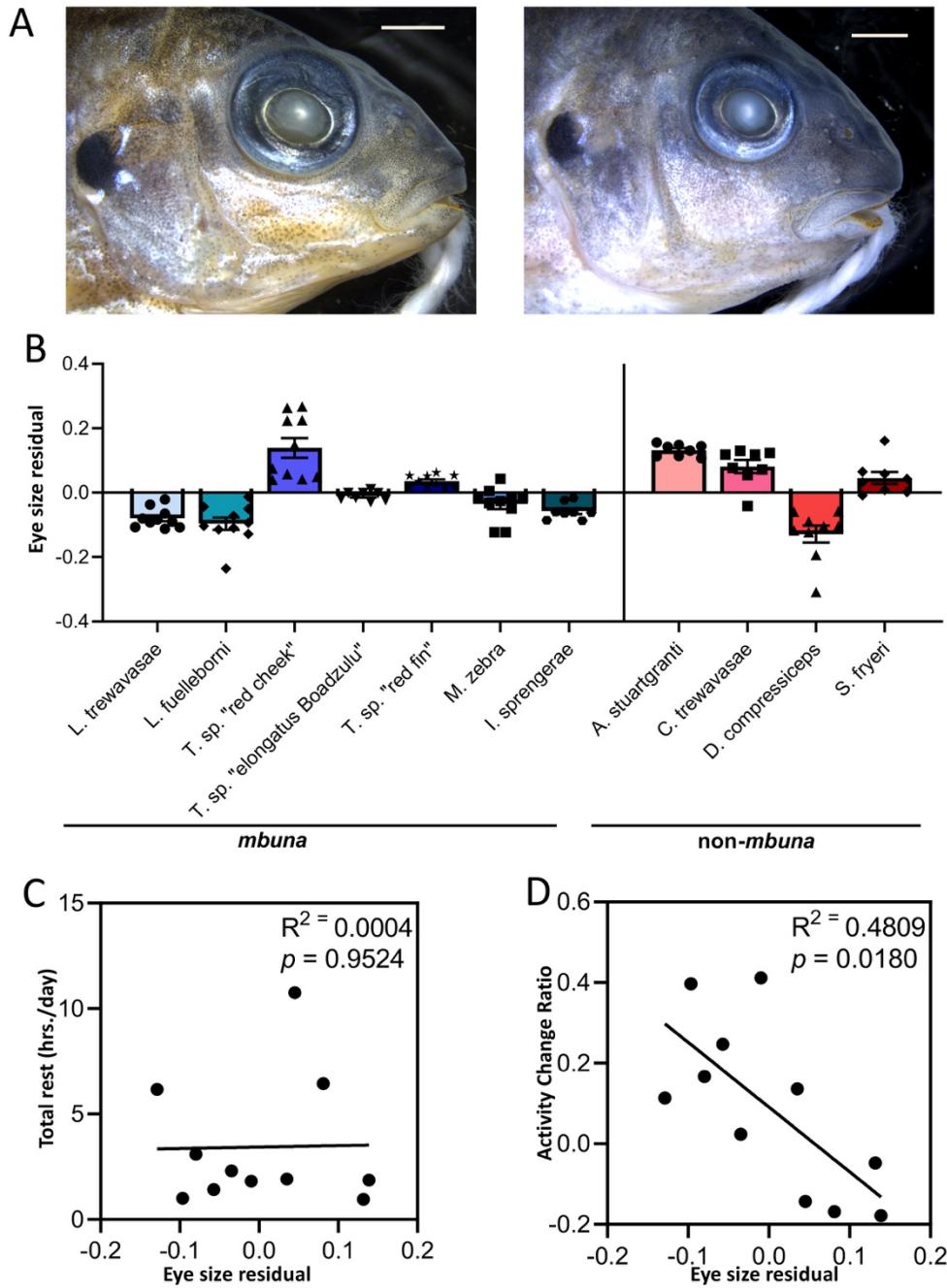
596

597 **Fig 3.** Presence or absence of shelter does not affect the nocturnal phenotype of *T. sp.*
598 "red cheek". (A) Select cichlid species were tested for locomotor activity over 24 hours
599 in the presence or absence of a 3 inch PVC tube, providing the option to take shelter at
600 any point during the day. (B) *T. sp. "red cheek"* maintain bias for nocturnal activity in the
601 presence of shelter (two-way ANOVA, $F_{2,12} = 7.9$, $p = 0.0065$). (C) *L. trewavasae* exhibit
602 significantly greater preference for the shelter relative to other species tested, consistent
603 with knowledge of the species' ecological niche (one-way ANOVA, $F_{2,12} = 6.305$, $p =$
604 0.0134). (D) Preference for shelter increases at night only in *L. trewavasae* (two-way
605 ANOVA, $F_{2,12} = 7.9$, $p = 0.0065$).



606

607 **Fig 4.** Locomotor activity in *T. sp. "red cheek"* is modulated by the presence/absence of
608 light. (A) Activity profiles of *T. sp. "red cheek"* under a 14:10 LD cycle (half-shaded
609 triangles), and in constant darkness (fully shaded triangles). (B) Under a 14:10 LD
610 cycle, *T. sp. "red cheek"* increase activity during the day; in 24 hours of darkness,
611 activity remains elevated throughout the 24-hour period (two-way ANOVA, $F_{1, 20} = 7.393$,
612 $p = 0.0132$).
613



614

615 **Fig 5.** (A) Photographs demonstrating difference in relative eye size between *T. sp. "red*
616 *cheek*", left, and *L. fuelleborni*, right. Both specimens were approximately 4 months old.
617 Scale bars equal 2mm. (B) Variation in eye size across species. (C) There is no
618 relationship between eye size and total rest amount. (D) There is a significant negative
619 relationship between eye size and a bias towards daytime activity.
620